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IS 15231 (2002): Anilophos + 2, 4-D Ethyl Ester EC [FAD 1: Pesticides and Pesticides Residue Analysis]

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ऐनीलोफोस + 2, 4-D ईथाइल ईसटर ई सी — विशिष्टि

Indian Standard

ANILOPHOS + 2, 4-D ETHYL ESTER EC — SPECIFICATION

ICS 65.100.20

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BUREAU OF INDIAN STANDARDS
MANAK BHAVAN, 9 BAHADUR SHAH ZAFAR MARG
NEW DELHI 110002

December 2002

Price Group 3

Pesticides Sectional Committee, FAD 1

FOREWORD

This Indian Standard was adopted by the Bureau of Indian Standards, after the draft finalized by the Pesticides Sectional Committee had been approved by the Food and Agriculture Division Council.

Anilophos + 2, 4-D ethyl ester EC is used as herbicide.

Anilophos + 2, 4-D ethyl ester is available in concentration containing anilophos 24 percent (*m/m*) and 2,4-D ethyl ester 32 percent (*m/m*), having 28.4 percent (*m/m*), having 2, 4-D acid.

In the preparation of this standard due consideration has been given to the provisions of *Insecticides Act*, 1968 and the Rules framed thereunder. However this standard is subject to restrictions imposed under the Act and Rules wherever applicable.

For the purpose of deciding whether a particular requirement of this standard is complied with, the final value, observed or calculated, expressing the result of a test or analysis, shall be rounded off in accordance with IS 2 : 1960 'Rules for rounding off numerical values (*revised*)'. The number of significant places retained in the rounded off value should be the same as that of the specified value in this standard.

Indian Standard

ANILOPHOS + 2, 4-D ETHYL ESTER EC — SPECIFICATION

1 SCOPE

This standard prescribes the requirements and methods of sampling and test for anilophos + 2, 4-D ethyl ester EC.

2 REFERENCES

The following standards contain provisions which through reference in this text, constitute provisions of this standard. At the time of publication, the editions indicated were valid. All standards are subject to revision, and parties to agreements based on this standard are encouraged to investigate the possibility of applying the most recent editions of the standards indicated below:

IS No.	*Title*
1070 : 1992	Reagent grade water (*third revision*)
1448 [P : 20] : 1998	Methods of test for petroleum and its products : [P : 20] Determination of flash point by Abel apparatus (*second revision*)
6940 : 1982	Methods of test for pesticides and their formulations (*first revision*)
7233 : 1991	Pesticide — 2, 4-D ethyl ester, technical — Specification (*second revision*)
8190 (Part 2) : 1988	Requirement for packing of pesticides : Part 2 Liquid pesticides (*second revision*)
9992 : 1991	Round and rectangular tin plate cans for liquid pesticides (*first revision*)
10627 : 1983	Method for sampling of pesticides formulations
13123 : 2000	Packing of liquid pesticides — Polyethylene terephthalate, (PET) bottles (up to 5 litre capacity) (*first revision*)
13402 : 1992	Pesticide — Anilophos, technical — Specification

3 REQUIREMENTS

3.1 Constituents

3.1.1 The material shall consist of anilophos technical + 2,4-D ethyl ester technical dissolved in suitable solvent(s) together with emulsifying agent(s) and with or without stabilizer(s).

3.1.2 Anilophos technical, employed in the manufacture of the material, shall conform to IS 13402.

3.1.3 2, 4-D ethyl ester technical, employed in the manufacture of the material, shall conform to IS 7233.

3.2 Physical

3.2.1 *Description*

The material shall be a brown coloured stable liquid, some turbidity/suspended matter may be present. It shall readily form an emulsion on dilution with water, suitable for spray.

3.2.2 *Cold Test*

No turbidity or separation of solid or oily matter shall occur when the material is subjected to the cold test at 10°C as prescribed in **13.1** of IS 6940.

3.2.3 *Flash Point* (*Abel*)

When determined by the method prescribed in IS 1448 [P : 20], the flash point of the material shall not be below 24.5°C.

3.2.4 *Emulsion Stability*

Any separation including creaming at the top and sedimentation at the bottom of 100 ml of emulsion prepared in standard hard water with 2.0 ml of EC, shall not exceed 2.0 ml when tested by the method prescribed in **13.3** of IS 6940.

3.3 Chemical

The material shall comply with the chemical requirements specified in **3.3.1** and **3.3.2**.

3.3.1 *Anilophos + 2, 4-D Ethyl Ester Content*

When determined by the method prescribed in Annex A and Annex B or by referee method, Annex C the observed anilophos content (*m/m*) and 2,4-D ethyl ester content (*m/m*) of any of the samples shall not differ from the declared nominal value by more than the tolerance limits given below:

Nominal Value, Percent	*Tolerance,* Percent	
Up to 9	+ 10 – 5	of the nominal value
Above 9 and below 50	± 5	
50 and above	+ 5 – 3	

3.3.1.1 Anilophos content + 2, 4-D ethyl ester content may be determined by Annex A and Annex B respectively (reverse phase method), and Annex C (normal phase), by high performance liquid chromatography. However the method prescribed in Annex C shall be used as referee method in case of dispute.

3.3.2 *Acidity/Alkalinity*

When tested by the method prescribed in **13.5** of IS 6940, the acidity (as H_2SO_4) or alkalinity (as NaOH) of the material shall be not more than 0.5 and 0.2 percent by mass respectively.

4 PACKING

The material shall be packed in containers as mentioned in IS 8190 (Part 2). The material shall be packed in either round tin containers lacquered from inside conforming to IS 9992 and or PET containers conforming to IS 13123 which shall be further packed in corrugated fiberboard boxes.

5 MARKING

5.1 The containers shall be securely closed and shall bear legibly and indelibly the following information in addition to any other information as is necessary under the *Insecticides Act*, 1968 and Rules framed thereunder:

a) Name of the material;
b) Indication of the source of manufacture;
c) Batch number;
d) Date of manufacture;
e) Date of expiry;
f) Net mass of contents;
g) Nominal anilophos content, percent (*m/m*); and nominal 2,4-D ethyl ester content, percent (*m/m*); and
h) A cautionary notice worded as in the *Insecticide Act*, 1968 and Rules framed thereunder; and *Standards of Weights and Measures (Packaged Commodities) Rules*, 1977.

5.2 BIS Certification Marking

The product may also be marked with the Standard Mark.

5.2.1 The use of the Standard Mark is governed by the provisions of the *Bureau of Indian Standards Act*, 1986 and the Rules and Regulations made thereunder. The details of conditions under which the licence for the use of the Standard Mark may be granted to manufacturers or producers may be obtained from the Bureau of Indian Standards.

6 SAMPLING

When freshly manufactured material in bulk quantity and/or the retail pack of the formulated product is/are offered for inspection, representative sample of the material shall be drawn and tested as prescribed in IS 10627 and if tested within 90 days of its date of manufacture, the criteria for conformity shall be the contents in percent (*m/m*), shall not be less than the declared nominal value. The upper limit for conformity shall be the same as those given in **3.3.1** of this standard.

When the material is offered for inspection after 90 days of its manufacture, sampling shall be done as prescribed in IS 10627, however, the criteria for conformity of the material, when tested, shall be the limits of tolerances, as applicable over the declared nominal value and given in **3.3.1**.

7 TESTS

7.1 Test shall be carried out by the appropriate method referred in **3.2** and **3.3**.

7.2 Quality of Reagents

Unless specified otherwise, pure chemicals and distilled water (*see* IS 1070) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities, which affect the results of analysis.

ANNEX A

(*Clause* 3.3.1)

DETERMINATION OF ANILOPHOS CONTENT

A-1 GENERAL

Anilophos content may be determined by reverse phase High Performance Liquid Chromatography (HPLC).

A-2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

A-2.1 Principle

A high performance liquid chromatography unit with an ultra-violet (U-V) detector is used for the determination. Using solutions containing known amounts of anilophos reference standard and internal standard, the response factor (R_f) for anilophos in the internal standard is arrived. A solution containing known mass of the anilophos sample and internal standard is injected subsequently. The percentage of anilophos in the sample is then computed by the standard relationship.

A-2.2 Apparatus

A-2.2.1 HPLC unit equipped with a U-V detector and a printer-plotter-cum-integrator. The HPLC operating conditions as suggested below can be changed in the equipment employed, provided standardization is done:

Column : Stainless steel, 15 cm length and 4.6 mm ID: packed with silica 5 µm coated with RP-C18 (octa-decylsilane), Lichrosphere, Merck. Cat. No. : 1.50823 or equivalent
Detector : Ultra-violet (230 nm)
Solvent system : Methanol : Water (70 : 30)
Solvent flow rate : 1.8 ml/min
Sample size : 20 ul

A-2.3 Reagents

A-2.3.1 *Solvent System* — Mix 70 percent methanol and 30 percent water (70 : 30).

A-2.3.2 *Anilophos Reference Standard* — of known purity.

A-2.3.3 *Internal Standard* — Nitrobenzene AR grade.

A-2.3.4 *Methanol* — HPLC grade or equivalent.

A-2.3.5 *Water* — HPLC grade or equivalent.

A-2.4 Procedure

A-2.4.1 *Preparation of Internal Standard Solution*

Weigh accurately (to the nearest 0.1 mg) approximately 50 mg of nitrobenzene into a 50-ml volumetric flask. Add few ml of methanol shake the flask to ensure the dissolution and make upto the mark with methanol.

Take 1 ml of this solution with a pipette in 10 ml of volumetric flask and make up with methanol. Homogenize.

A-2.4.2 *Preparation of Reference Standard Solution*

Weigh accurately (to the nearest 0.1 mg) approximately 50 mg of anilophos reference standard into a 50 ml volumetric flask. Add few ml of methanol, shake well to ensure the dissolution and make up the volume with methanol.

Pipette out 1 ml of this solution and 1 ml of internal standard solution into 10 ml volumetric flask and make upto the mark with methanol. Homogenize.

A-2.4.3 *Preparation of Sample Solution*

Weigh accurately to the nearest 0.1 mg approximately 100 mg of sample in a 50 ml of volumetric flask, add few ml of methanol in it, and shake well to ensure the dissolution. Make upto the mark with methanol.

Take out 1 ml of this solution and 1 ml of internal standard solution in 10 ml of volumetric flask and make up to the mark with methanol. Homogenize.

A-2.4.4 *Estimation*

A-2.4.4.1 Inject 20 µl anilophos reference standard solution into the HPLC unit, and from the chromatogram, calculate the response factor (R_{f1}) as follow. Repeat the injection till R_{f1} values agree well within ± 2 percent.

$$\text{Response factor } (R_{f2}) = \frac{A_1}{A_2}$$

where

A_1 = peak area for the anilophos in chromatogram of reference standard, and
A_2 = peak area for the internal standard in chromatogram of standard.

A-2.4.4.2 Introduce 20 µl sample solution into the HPLC unit and calculate the response factor (R_{f2}) as described in **A-2.4.4.1**.

$$\text{Response factor } (R_{f2}) = \frac{A_3}{A_4}$$

where

A_3 = peak area for the anilophos in chromatogram of sample, and
A_4 = peak area for the internal standard in chromatogram of sample.

A-2.5 Calculation

$$\text{Anilophos content, percent by mass} = \frac{R_{f2} \times M_1}{R_{f1} \times M_2} \times P$$

where

R_{f2} = response factor for anilophos sample;
M_1 = mass, in mg of anilophos reference;
R_{f1} = response factor for reference standard;
M_2 = mass, in mg of anilophos sample; and
P = percent purity of anilophos reference standard.

ANNEX B

(*Clause* 3.3.1)

DETERMINATION OF 2,4-D ETHYL ESTER CONTENT

B-1 GENERAL

2,4-D ethyl ester content may be determined by hydrolysis of ester to acid in presence of methanolic potassium hydroxide solution and 2, 4-D acid is determined by reverse phase high performance liquid chromatography.

B-2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

B-2.1 Principle

A high performance liquid chromatography unit with an ultra-violet (U-V) detector is used for the determination. Using solutions containing known amounts of 2,4-D ethyl ester reference standard and internal standard, the response factor (R_f) for 2,4-D ethyl ester is arrived. A solution containing known mass the 2,4-D ethyl ester sample and internal standard is injected subsequently. The percentage of 2, 4-D ethyl ester in the sample is then computed by the standard relationship.

B-2.2 Apparatus

B-2.2.1 A HPLC unit equipped with a U-V detector and a printer-plotter-cum-integrator. The operating condition as suggested below can be changed in the equipment employed, provided standardization is done:

Column : Stainless steel, 25 cm length and 4.6 mm ID packed with silica 5 µm particle size coated with RP- C18 (octa-decylsilane) Lichrosphere, Merck. Cat. No. :.1.50833 or equivalent

Detector : Ultra-violet (230 nm)

Solvent system : Methanol : Water (75 : 25)

Solvent flow rate : 1.5 ml/min

Sample size : 20 µl

B-2.3 Reagents

B-2.3.1 *2,4-D Ethyl Ester Reference Standard* — of known purity.

B-2.3.2 *Internal Standard — Dibutyl Phthalate* — AR grade or equivalent.

B-2.3.3 *Methanol* — HPLC grade or equivalent.

B-2.3.4 *Water* — HPLC grade or equivalent.

B-2.3.5 *Methanolic Potassium Hydroxide* — 0.02 N.

B-2.3.6 *Solvent System* — Mix 75 percent methanol and 25 percent water (75 : 25).

B-2.4 Procedure

B-2.4.1 *Preparation of Internal Standard Solution*

Weigh, accurately to the nearest 0.1 mg, 50 mg of Dibutyl phthalate into a 50 ml volumetric flask, add small quantity of methanol, mix well to ensure the dissolution and make upto the volume with methanol.

Take 10 ml of this solution and 20 ml of methanol in another volumetric flask, mix well.

B-2.4.2 *Preparation of Reference Standard Solution*

Weigh accurately to the nearest 0.1 mg, 100 mg of 2, 4-D ethyl ester reference standard into a 25 ml volumetric flask and make upto the mark with methanol. Homogenize.

Take 5 ml of this solution into a 25 ml volumetric flask, add 15 ml of 0.02 N methanolic potassium hydroxide and shake well. Reflux the mixture at 60°C for 20 min.

Cool the mixture at room temperature. Make the volume to 25 ml with 0.02 N methanolic potassium hydroxide. Homogenize.

Take out 1 ml of this solution and 4 ml of internal standard solution into a 50 ml of volumetric flask. Shake well and make upto the mark with methanol. Homogenize.

B-2.4.3 *Preparation of Sample Solution*

Weigh accurately nearest to the 0.1 mg approximately 325 mg of sample into a 25 ml of volumetric flask. Make upto the mark with methanol. Homogenize. Take out 5 ml of this solution into a 25 ml of volumetric flask, add 15 ml of 0.02 N methanolic potassium hydroxide and shake well. Reflux the mixture at 60°C for 20 min. Cool the mixture at room temperature. Make the volume with 0.02 N methanolic potassium hydroxide.

Take out 1 ml of this solution and 4 ml of internal standard solution into a 50 ml of volumetric flask. Shake well and make upto the mark with methanol. Homogenize.

B-2.4.4 *Estimation*

B-2.4.4.1 Introduce 20 ul 2, 4-D ethyl ester reference standard solution into the HPLC unit, and from the chromatogram, calculate the response factor (R_{f1}) as follows. Repeat the injection till R_{f1} values agree well within ± 2 percent.

$$\text{Response factor } (R_{f1}) = \frac{A_1}{A_2}$$

where

A_1 = peak area for the 2, 4-D ethyl ester reference in chromatogram of standard, and

A_2 = peak area for the internal standard in chromatogram of standard.

B-2.4.4.2 Introduce 20 µl sample solution into the HPLC unit and calculate the response factor (R_{f2}) as described in **B-2.4.4.1**.

$$\text{Response factor } (R_{f1}) = \frac{A_3}{A_4}$$

where

A_3 = peak area for the 2,4-D ethyl ester in chromatogram of standard, and

A_4 = peak area for the internal standard in chromatogram of standard.

B-2.5 Calculation

2,4-D ethyl ester content, (expressed as total extractable 2, 4-D acid content), percent *m/m*, percent by mass $= \dfrac{R_{f2} \times M_1}{R_{f1} \times M_2} \times P \times \dfrac{221}{249}$

where

R_{f2} = response factor for the 2,4-D ethyl ester sample;

M_1 = mass, in mg of 2, 4-D ethyl ester reference;

R_{f2} = response factor for the reference standard;

M_2 = mass, in mg of 2, 4-D ethyl ester sample; and

P = percent purity of 2, 4-D ethyl ester reference standard.

ANNEX C

(*Clause* 3.3.1)

DETERMINATION OF ANILOPHOS AND 2, 4-D ETHYL ESTER CONTENT REFEREE METHOD (NORMAL PHASE)

C-1 GENERAL

Anilophos content + 2, 4-D ethyl ester content may be determined by normal phase high performance liquid chromatography.

C-2 HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

C-2.1 Principle

A high performance liquid chromatography unit with an ultra-violet (U-V) detector is used for the determination. Using solutions containing known purity of anilophos reference standard and 2,4-D ethyl ester and sample solution, comparing the response of both the active ingredients by HPLC on a column packed with spherical silica, for example E. Merck-Licrosphere Si_{60} 5 µm Cat. No. : 1.50830 or equivalent.

C-2.2 Apparatus

C-2.2.1 *High Pressure Liquid Chromatograph (HPLC)*

A HPLC unit equipped with a U-V detector and a printer-plotter-cum-integrator. The operating condition as suggested below can be changed in the equipment employed, provided standardization is done:

Column : Stainless steel, 25 cm long and 4.6 mm ID packed with speherical Silica 60, 5 µm, particle size-Licrosphere Si 60 5 µm Cat. No. 1.50830 or equivalent

Detector : Ultra-violet

Wave length : 254 nm

Solvent system : *Iso*-octane : 1,4 Dioxane (90 : 10 *v*/*v*)

Solvent flow rate : 1.5 ml/min

Typical retention time : 2, 4-D ethyl ester 5.0 min
Anilophos 17.0 min

Run time : 30 min

C-2.3 Reagents

C-2.3.1 *2,4-D Ethyl Ester Reference Standard* — of known purity.

C-2.3.2 *Anilophos Reference Standard* — of known purity.

C-2.3.3 Iso-*octane* — HPLC grade or equivalent.

C-2.3.4 *1,4 Dioxane* — HPLC grade or equivalent (saturated with 1.5 ml/ l of water).

C-2.3.5 *Methylene Chloride* — HPLC grade or equivalent.

C-2.3.6 *Mobile Phase* — Mix 900 ml *iso*-octane with 100 ml 1, 4 dioxane (90 : 10).

C-2.3.7 *Make-up Phase* — Mix 800 ml *iso*-octane and 200 ml 1, 4 dioxane (80 : 20).

C-2.4 Procedure

C-2.4.1 *Preparation of Reference Standard Solution*

Weigh, accurately (to the nearest 0.1 mg) 75 mg of anilophos reference standard and 100 mg of 2, 4-D ethyl ester reference standard into a 25 ml of volumetric flask . Add 4 ml of ethylene chloride in it and shake the flask to ensure the dissolution of the standard. Make upto the mark with make up phase.

Now, take 5 ml of this solution into another 25 ml volumetric flask. Make upto the mark with make up phase. Homogenize.

C-2.4.2 *Preparation of Sample Solution*

Weigh accurately to the nearest 0.1 mg of approximately 300 mg of sample into a 25 ml of volumetric flask. Add 4 ml of methylene chloride in it and shake the flask to ensure the dissolution. Make upto the mark with make up phase.

Now, take 5 ml of this solution into another 25 ml volumetric flask. Make upto the mark with make up phase. Homogenize.

C-2.4.3 *Estimation*

Inject 20 µl reference standard solution into the HPLC unit, until peak area of two successive injections agree to within 2 percent. Inject the standard and sample solution in succession according to the following sequences:

$$R_1S_1,\ R_2S_1,\ R_1S_2, \text{ and } R_2S_2$$

C-2.4.4 *Calculation*

$$\text{Anilophos content, percent by mass} = \frac{A_1 \times M_2}{A_2 \times M_1} \times P$$

where

A_1 = peak area of anilophos in the chromatogram of sample solution,

A_2 = peak area of anilophos in the chromatogram of standard reference solution,

M_1 = weight in mg of sample,

M_2 = weight in mg of anilophos standard reference, and

P = purity of anilophos standard reference.

$$\text{2, 4-D ethyl ester content percent by mass (expressed as total exctractable 2, 4-D acid), percent by mass} = \frac{A_3 \times M_4}{A_4 \times M_3} \times P \times \frac{221}{249}$$

where

A_3 = peak area of 2, 4-D ethyl ester in the chromatogram of sample solution,

A_4 = peak area of 2, 4-D ethyl ester in the chromatogram of standard reference solution,

M_3 = weight of sample in mg,

M_4 = weight of 2, 4-D ethyl ester standard reference in mg, and

P = purity of 2, 4-D ethyl ester standard reference.

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